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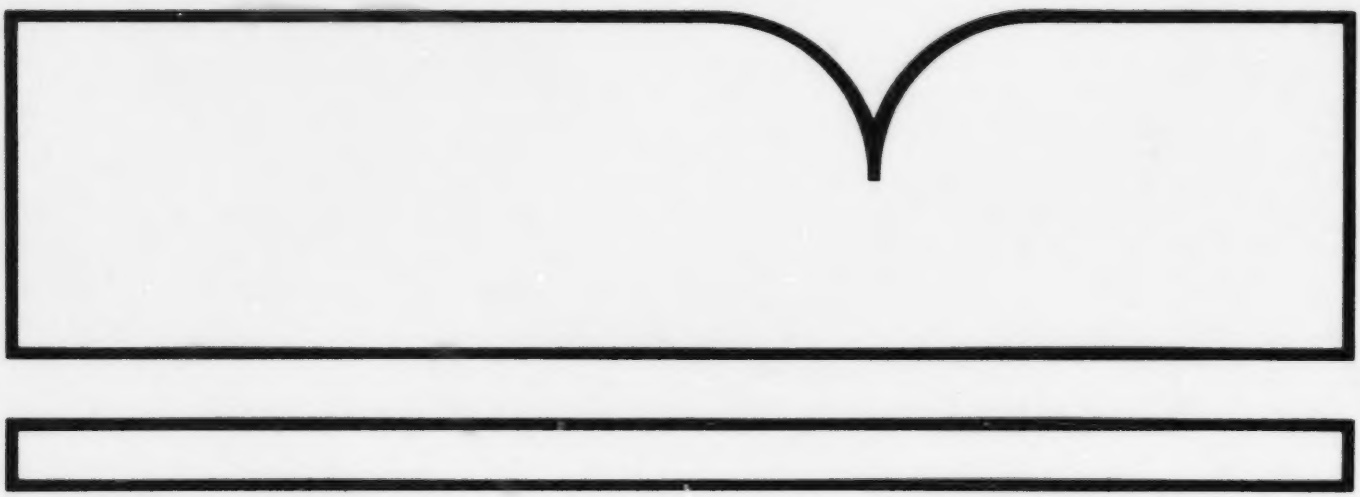

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INFLUENCE OF LINDANE ON SURVIVAL AND OSMOREGULATORY/METABOLIC RESPONSES OF THE LARVAE AND ADULTS OF THE ESTUARINE CRAB, EURYPANOPEUS DEPRESSUS

Michael A. Shirley and Charles L. McKenney, Jr.

ABSTRACT

Short-term exposure to sublethal concentrations of the organochlorine insecticide, lindane, caused alterations in ionic and osmotic regulatory abilities and related compensatory metabolic mechanisms in the xanthid crab Eurypanopeus depressus. A lindane exposure concentration of 1.45 µg/L reduced hemolymph osmotic concentrations in adult crabs; however, chloride ion regulation was more sensitive, being disrupted at a lindane exposure concentration of 0.70 µg/L. Larval stages proved to be more sensitive to lindane exposure than adults. A lindane exposure concentration of 0.01 µg/L increased larval mortality and altered larval respiration and ammonia excretion rates. Zoeae, megalopae and adults of the crab, E. depressus, appear to possess different response patterns to hypoosmotic stress and lindane exposure.

INTRODUCTION

Lindane, the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane, has continued significant commercial use as an insecticide in the United States (U.S.E.P.A., 1980). Acute toxicity tests with various marine organisms determined crustaceans to be the most sensitive to lindane. The pink shrimp (Penaeus duorarum) was the most sensitive species tested, with a LC50 value of 0.17 µg/L (Schimmel et al., 1977). Current federal regulations are based upon this acute toxicological data and suggest the "safe" ambient concentration of lindane for salt water aquatic life to be

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≤0.16 µg/L. (U.S.E.P.A., 1980). The purpose of this investigation was to provide some information regarding the effects of chronic lindane exposure on a marine crustacean.

The crustacean chosen as the experimental organism during this study was the xanthid crab Eurypanopeus depressus, one of the most abundant mud crabs found in estuarine oyster beds. This crab has a broad distribution range in the U.S., extending from Massachusetts through Texas, and has been found in the intertidal zone to 50 m and in salinities of 4 o/oo and above (Ryan, 1956; Williams, 1965; McDonald, 1977). Since all larval stages of this species are found within estuarine waters (Pinschmidt, 1963), it would be continuously exposed to estuarine pollutants throughout metamorphosis.

For estuarine organisms, such as E. depressus, the ability to adjust to salinity changes is a primary adaptation to their environment. Short-term osmoregulatory responses to changing salinity require adjustments in gill membrane permeability (Ruddy & Feng, 1975), ion transport capabilities (Towle, 1981), and intracellular amino acid metabolism (Mangum et al., 1976). Any factor which interfers with these osmotic adaptations limits the capacity of these organisms to adjust to changing salinity and reduces their probability for survival (Jones, 1973, 1975). As such, deviations in these adaptive internal osmotic adjustments have been suggested as a potentially useful biological parameter for assessing stress in estuarine biota (Lockwood, 1979; Bayne et al., 1980; Sastry & Miller, 1980).

Lipophilic organochlorine toxicants have been shown to affect membrane-mediated processes. Lindane's lethal effect on insects is thought to be due to impaired neural transmission (Corbett, 1974; O'Brien, 1967; Mueller et al., 1981). Gills are a major route of organochlorine uptake in aquatic crustaceans, therefore, disruption of gill membrane-mediated processes, such as osmoregulation, may represent the primary mode of action of organochlorine compounds in aquatic species.

Gill Na^+,K^+-ATPase activity in several marine crab species was inhibited by exposure to organochlorines, including DDT (Neufeld & Pritchard, 1979) and methoxychlor (Caldwell, 1974). Callinectes sapidus showed a temporary osmotic response to DDT (Neufeld & Pritchard, 1978). Matsumura (1972) demonstrated that the toxicity of lindane to the brine shrimp, Artemia salina, varied as the NaCl concentration changed. A direct link was established between lindane exposure and sodium regulation in the amphipod Gammarus

duebeni; short-term exposure to sublethal concentrations of lindane in low salinities resulted in significant reductions in hemolymph sodium concentrations (Inman & Lockwood, 1977).

The objectives of the present research were 1) to compare acute and chronic toxicity to lindane by larval and adult Eurypanopeus depressus and 2) to examine any alterations in short-term osmoregulatory responses and compensatory metabolic mechanisms of larval and adult E. depressus when transferred to hypoosmotic conditions after sublethal exposure to lindane. Larval acute- and chronic-toxicity values are compared with adult values to examine the relative sensitivities of these different life stages to lindane exposure. Adult hemolymph osmotic regulation and chloride ion regulation were measured to assess any lindane-induced disruption of short-term extracellular osmoregulatory mechanisms. Ammonia excretion and oxygen consumption rates of lindane-exposed larvae and adults were used to examine the metabolic responses of these life stages to hypoosmotic salinities after lindane exposure.

MATERIALS AND METHODS

Collection, Culture, and Maintenance of Animals

Adult Eurypanopeus depressus were collected from oyster beds located on the Eglin Air Force Base Reserve, Fort Walton Beach, Florida. Animals were maintained at the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida, in culture tanks receiving continuously flowing, filtered (20 μm) natural seawater of fluctuating salinity and temperature. These animals were fed frozen adult Artemia. Gravid females were removed and placed in Carolina culture bowls under controlled temperature (25 ± 2°C) and salinity (27 ± 2 o/oo) conditions. Water was replaced daily. Larvae were obtained randomly by mixing at least three broods of released offspring. Only larvae exhibiting active, positive photoactic behavior were used. Larvae were fed 24-h-old Artemia nauplii.

Exposure System

Test tanks were separated into three replicate chambers receiving continously flowing, filtered (20 μm) seawater with controlled salinity (27 ± 2 o/oo) and temperature (25 ± 2°C) conditions. A flow rate of 5 x volume/24 h was maintained for each replicate chamber. Temperature and salinity

were recorded daily. Dissolved oxygen concentrations were recorded at the start of each study and then weekly thereafter. Aeration of the water prior to entering the test tank and the flow rate through the tanks were adequate to maintain dissolved oxygen (DO) levels consistently above 6.4 ppm.

Animals were held in 1000-mL glass beakers which had four one-inch diameter holes drilled through the sides near the bottom. The holes were covered with 150-μm Nitex® screening. Three adults crabs or twenty larvae were enclosed in each beaker for both the acute and chronic studies.

Triethylene glycol and acetone served as carriers for lindane in studies with adults and larvae, respectively. Triethylene glycol concentrations did not exceed 12 μg/L and acetone concentrations did not exceed 4 μg/L. The carrier and carrier-lindane mixtures were introduced via syringe pumps. A tank receiving the carrier-seawater mixture served as the carrier control, whereas, a tank receiving only seawater served as a seawater control. Lindane concentrations were measured on Day 1 and Day 4 of the 96-h studies, while weekly samples of exposure water were analyzed at the start and then once weekly during the 21-d study. Gas-liquid chromatography was used for this analysis (Schimmel et al., 1977).

Acute and Chronic Survival

Adult mortality was determined daily over both the 96-h period for the acute toxicity study and during the 21-d study. Each exposure tank contained 9 animals/replicate chamber for a total of 27 animals/exposure concentration. As death occured, animals were removed from the exposure system.

Each larval exposure tank contained 60 animals/replicate chamber for a total of 180 animals/exposure concentration. Survival of larvae from hatching to Day 4 was observed daily during the 96-h acute toxicity study. Survival during the chronic studies was monitored weekly.

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Metabolic Measurements

On days corresponding with the intermolt of stage IV zoeae (Day 16) and megalopae (Day 21) for the larval study or on Day 21 of the lindane exposure period for the adult study, 16 animals from each exposure level of lindane were sealed individually in all-glass syringes (volume = 5 ml for larvae or 30 ml for adults) containing fully oxygenated lindane-free seawater of either 10 or 27 o/oo salinity. For each set of eight crabs for a particular salinity-lindane treatment, two syringes with no animals served as controls. All syringes were placed in a water bath at 25°C.

After approximately 4 h incubation period for larvae and 1 h for adults, a 75-100 µl sample of fluid from each syring was injected into a Radiometer BM53 MK2 Blood Microsystem with attached PHM71 MK2 Acid-Base Analyzer and direct measurement recorded for partial pressure of oxygen. Another 100 µl of sample was injected into a micro flow-through cap (Model 95-00-25) attached to an Orion Ammonia Electrode (Model 95-10) connected to an Orion Research Microprocessor Ionalyzer (Model 901). Larvae were then removed from the syringe, briefly rinsed with distilled water, and placed in an oven at 60°C to dry for 48 h. Dry weights of individual animals were measured to the nearest 0.1 µg on a Cahn Automatic Electrobalance. Adults remained in the syringes in the water bath until just prior to removal of hemolymph samples for osmotic concentration and chloride ion determinations, which followed as soon as possible after metabolic measurements. They were then rinsed and dried as above. Weights were determined to the nearest 0.1 mg.

Differences between partial pressures of oxygen and ammonia concentrations in the control syringes with no animals and syringes containing an animal were attributed to respiration and ammonia excretion, respectively. These values were corrected for the weight of the animal and the time in the respirometer, and both weight-specific ammonia excretion rates (µg NH_3-N·mg dry weight^{-1}·h^{-1}) and weight-specific oxygen consumption rates (µg O_2·mg dry weight^{-1}·h^{-1}) were calculated.

Hemolymph Analysis

To determine hemolymph osmoregulatory responses of adult Eurypanopeus depressus, adult crabs were placed in a range of salinities from 10 to 31 o/oo. Osmotic concentrations of hemolymph samples from these crabs were determined

24 and 48 h after transfer to the various salinities by the procedure described below. For lindane-exposed crabs, two 5-µl hemolymph samples were withdrawn from each adult E. depressus as rapidly as possible after metabolic measurements. The hemolymph samples were taken by capillary action via a Drummond 5-µl microcap from a hole punctured in the ventral medial surface of the carapace. One sample was analyzed for osmotic concentration using a Wescor Vapor Pressure Osmometer (Model 5100 C) and the other for chloride ion using a Buchler Model 4-2500 Digital Chloridometer. Chloride ion ratios were calculated as hemolymph chloride ion concentration to environmental chloride ion concentration.

Statistics

The 96-h LC50 values for lindane exposure were calculated by the probit and binomial methods with Abbott's correction for control mortality (Finney, 1971). Response patterns of adults and larvae (megalopae or zoeae) maintained in isosmotic salinity or transferred to hypoosmotic salinity after exposure to one of three lindane concentrations were analyzed by a 2 x 3 and 2 x 2 x 3 factorial analysis of variance (ANOVA), respectively. Before these analyses, Hartley's test for homoscedasticity (Neter & Wasserman, 1974) was used to test for equality of variances. Treatment response means were compared using the Student-Neuman-Keul (SNK) post-hoc test at alpha levels of 0.05 and 0.01.

RESULTS

Acute and Chronic Toxicity

The 96-h LC50 value of lindane for adult Eurypanopeus depressus crabs was determined to be approximately 25 µg/L. Stage I zoeae were more sensitive to lindane exposure, with a 96-h LC50 value of approximately 0.66 µg/L. Adult mortality increased proportionally to increasing lindane exposure concentrations; however, larval mortality did not differ at lindane exposure concentrations of 0.01 and 0.11 µg/L.

During the 21-d exposure for adult crabs, lindane exposures of 0.70 and 1.45 µg/L did not increase adult crab mortality relative to the control (Table 1) (ANOVA, $\alpha = 0.05$).

Table 1. Survival percentage (mean ± SE) of adult and larval Eurypanopeus depressus exposed to a range of measured concentrations of lindane.

Life Stage	Lindane Exposure Concentration (µg/L)	Duration of Exposure (days)	Percentage Survival
Adult	0.00	21	67 ± 6.4
	0.70	21	70 ± 12.7
	1.45	21	67 ± 11.0
Hatch to zoea IV	0.00	16	75 ± 5.0
	0.01	16	63 ± 16.1
	0.11	16	68 ± 5.7
	1.08	16	0* ± 0.0
Hatch to megalopa	0.00	21	73 ± 6.6
	0.01	21	27* ± 2.5
	0.11	21	41* ± 3.7

*Significantly less than control ($\alpha = 0.05$)

The survival of larvae reared to zoeal stage IV with continual exposure to lindane concentrations of 0.01 and 0.11 µg/L was not statistically different ($\alpha = 0.05$) from the control. However, a lindane exposure concentration of 1.08 µg/L resulted in 100% larval mortality before development to zoeal stage IV (Table 1). Lindane exposure concentrations of 0.01 and 0.11 µg/L produced significant larval mortality during development to the megalopal stage.

Osmoregulation

The osmoregulatory curve of adult Eurypanopeus depressus is depicted in Figure 1. Since no significant differences were found between osmotic responses of adult crabs after 24- or 48-h exposure to a new experimental salinity, responses from both exposure periods were combined in the curve. Adult crabs demonstrated hyperosmoregulation at low salinities and osmoconformity at high salinities, with an isosmotic point of approximately 27 ± 2 o/oo S.

Exposure to a lindane concentration of 1.45 µg/L decreased the hyperosmoregulatory ability of crabs transferred to low salinity (Fig. 2). A 2 x 3 factorial analysis

of variance of osmotic concentration of adult hemolymph revealed significant ($\alpha = 0.05$) effects due to salinity transfer or lindane exposure and a significant interaction between these two treatment factors. The SNK procedure indicated a significant ($\alpha = 0.05$) reduction in hemolymph osmotic concentration of animals transferred to 10 o/oo salinity after exposure to a lindane concentration of 1.45 µg/L.

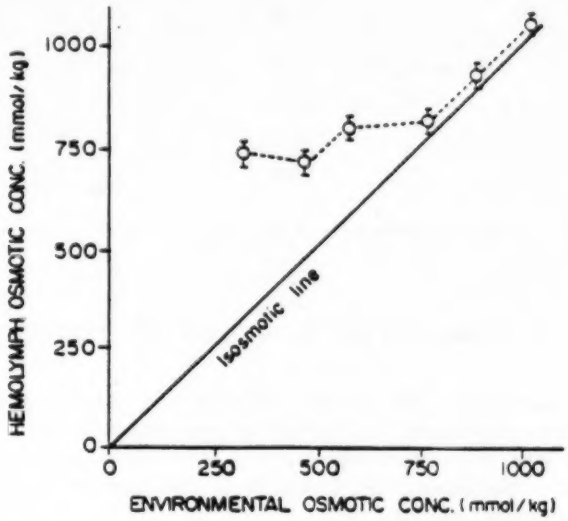

Fig. 1. Osmoregulatory curve for adult Eurypanopeus depressus. Values represent mean ± SD.

Chloride ion ratios of adult crabs subjected to lindane exposure and hypoosmotic stress are shown in Figure 3. Statistical analyses showed that chloride ion ratios were significantly (ANOVA, $\alpha = 0.01$) altered by both salinity and lindane exposure. Interaction between the two treatments was also significant at this alpha level. Upon transfer to low salinity, lindane exposure resulted in chloride ion ratios which approached unity. The SNK procedure indicated that prior exposure to lindane significantly ($\alpha = 0.01$) reduced the chloride ion ratios of crabs transferred to low salinity. This reduction was found to be dose dependent. At high salinity, the chloride ion ratios of lindane-exposed crabs did not differ from those of the control animals.

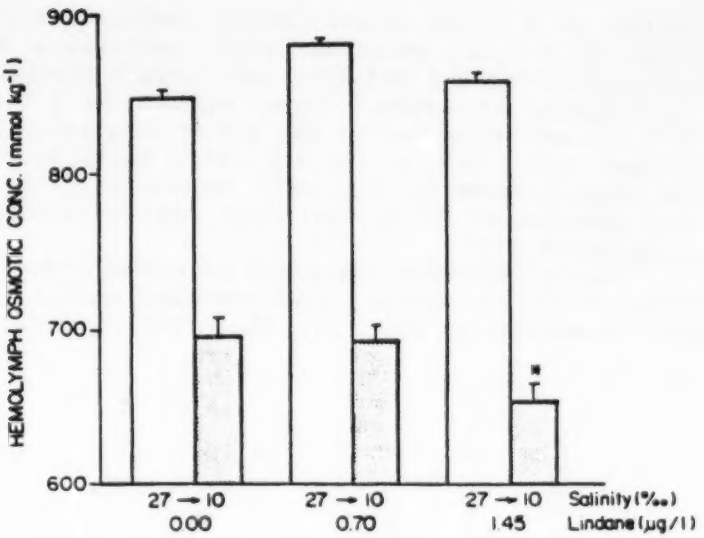

Fig. 2. Hemolymph osmotic concentrations of adult Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 21-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A single asterick indicates a significant difference (α = 0.05) from the control at that salinity.

Metabolic Responses

There was an observable trend toward a dose-dependent decrease in adult respiration rate with lindane exposure (Fig. 4). Lindane exposure significantly (ANOVA, α = 0.01) affected adult respiration rates. Salinity or lindane exposure-salinity interaction effects were not found to be significant. The results of a SNK comparison on respiration rate response means at each lindane concentration indicated that lindane exposure significantly (α = 0.01) reduced adult respiration rates. Using the SNK procedure, a lindane concentration of 0.70 µg/L produced a significant effect at an alpha level of 0.05, but not at 0.01; however, a lindane exposure concentration of 1.45 µg/L was found to significantly reduce respiration rates even at the lower alpha level.

In general, respiration rates of zoeae and megalopae increased with lindane exposure at high salinity (Figs. 5 & 6). Lindane exposure or salinity was found to significantly

(ANOVA, $\alpha = 0.01$) affect larval respiration rates. The ANOVA ($\alpha = 0.05$) procedure also indicated a significant interaction between salinity and lindane exposure effects. Low-salinity transferred zoeae exposed to a 0.01 or 0.11 µg/L lindane concentration had higher respiration rates than control zoeae (SNK, $\alpha = 0.01$). When those megalopae which had been exposed to a lindane concentration of 0.11 µg/L were transferred to low salinity, they exhibited increased respiration rates.

Lindane exposure was found to reduce ammonia excretion rates of adult crabs in a dose-dependent manner at both high and low salinities (Fig. 7). There were significant (ANOVA,

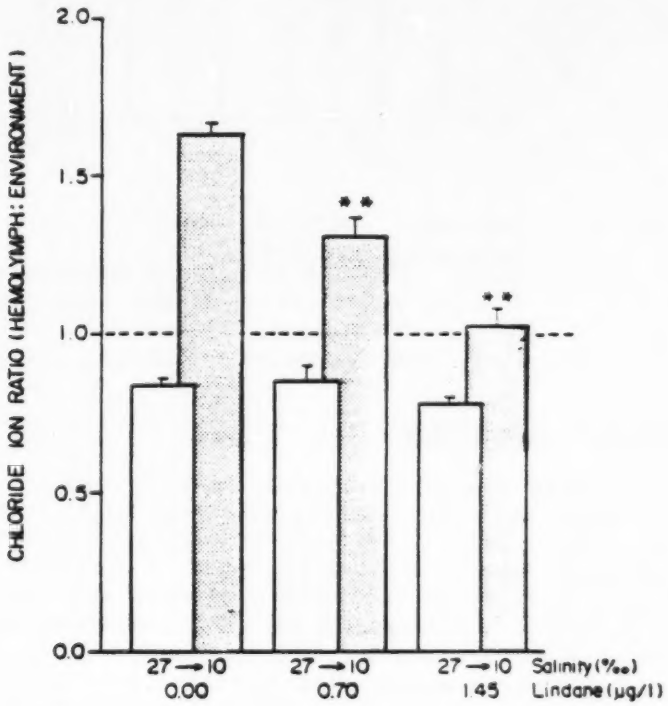

Fig. 3. Chloride ion ratios of adult Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 21-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A double asterick indicates a significant difference ($\alpha = 0.01$) from the control at that salinity.

α = 0.05) effects due to salinity transfer or lindane exposure but no interaction between treatment factors with respect to ammonia excretion rates. The SNK post-hoc test procedure demonstrated (α = 0.01) reduced ammonia excretion rates at isosmotic or hypoosmotic salinities for crabs which had been exposed to lindane.

Lindane exposure also reduced ammonia excretion rates of all larval stages at the isosmotic salinity (Figs. 8 & 9). However, salinity, lindane exposure, and larval stage were found to significantly (ANOVA, α = 0.01) affect larval ammonia excretion rates. The interaction effects between

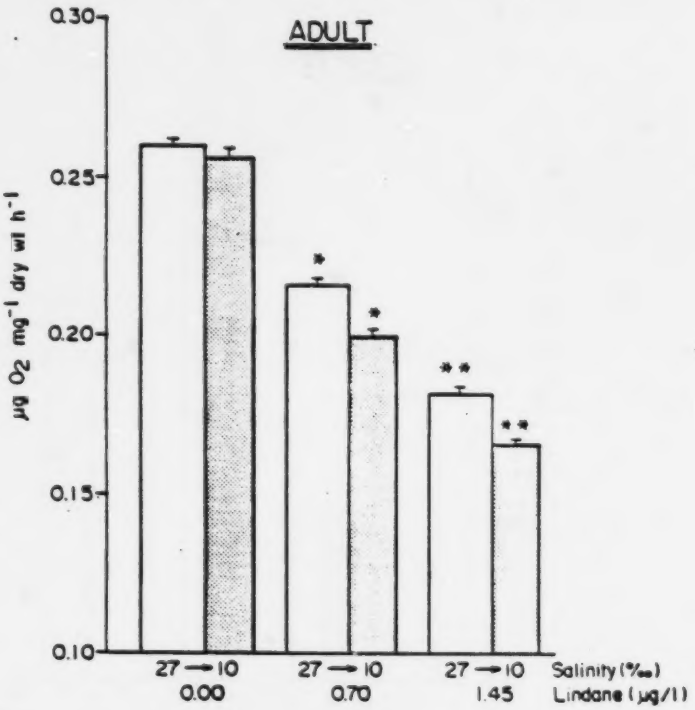

Fig. 4. Respiration rates of adult Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 21-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A single or double asterick indicates a significant difference (α = 0.05 or 0.01, respectively) from the control at that salinity.

these three factors were also found to be significant (ANOVA, $\alpha = 0.01$). Megalopae transferred to low salinity after exposure to lindane continued to exhibit a dose-dependent reduction in ammonia excretion rates (SNK, $\alpha = 0.01$). However, a lindane exposure of 0.11 µg/L did not affect ammonia excretion rates of low-salinity transferred zoeae. Whereas, a lindane exposure concentration of 0.01 µg/L significantly (SNK, $\alpha = 0.01$) increased the ammonia excretion rates of zoeae transfered to low salinity.

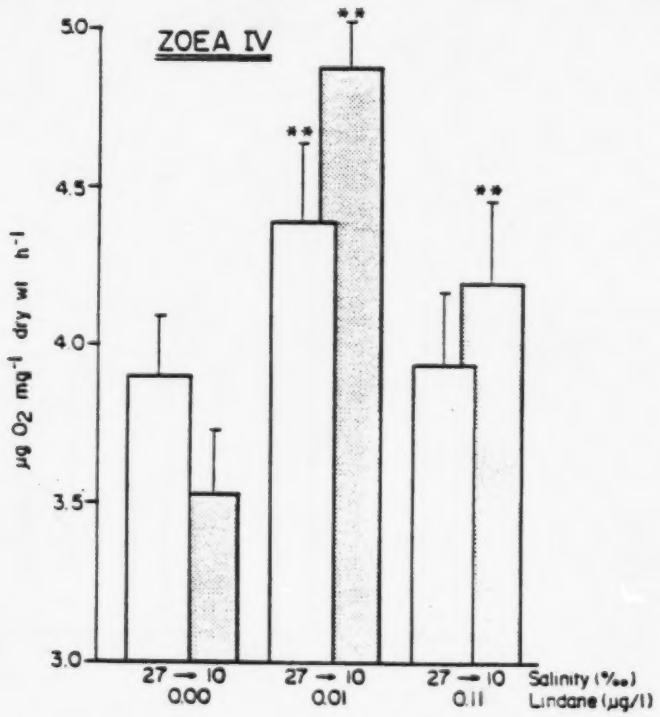

Fig. 5. Respiration rates of zoeal stage IV Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 16-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A double asterick indicates a significant difference ($\alpha = 0.01$) from the control at that salinity.

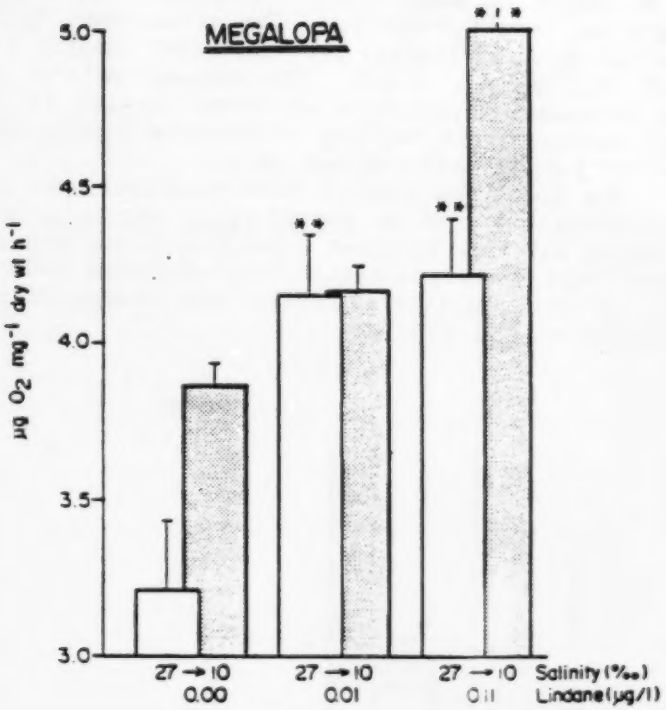

Fig. 6. Respiration rates of megalopal Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 21-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A double asterick indicates a significant difference (α = 0.01) from the control at that salinity.

DISCUSSION

Eurypanopeus depressus larvae, through the first 4 d of development, were more sensitive to lindane exposure than adults. The larval 96-h (hatch to zoea I) LC50 value for lindane exposure was 0.66 μg/L, as opposed to 25 μg/L for adult crabs. Although the former value is still somewhat higher than the 96-h LC50 of lindane for the pink shrimp Penaeus duorarum (0.17 μg/L; Schimmel et al., 1977), continuous exposure to a lindane concentration of 0.01 μg/L over

21 d (hatch to megalopa) resulted in a 46% increase in E. depressus larval mortality. The established "safe" ambient concentration of lindane for salt water aquatic life is 0.16 µg/L (U.S.E.P.A., 1980). The present results demonstrate the increased sensitivity of larval stages to lindane and the inadequacy of setting regulatory limits based solely upon 96-h acute-toxicity test data.

The hemolymph osmotic concentrations and chloride ion regulation patterns of Eurypanopeus depressus subjected to changing salinity typified a euryhaline osmoregulation. They showed hyperosmoregulation at low salinities and osmoconformity at high salinities, with the isosmotic point being approximately 27 ± 2 o/oo.

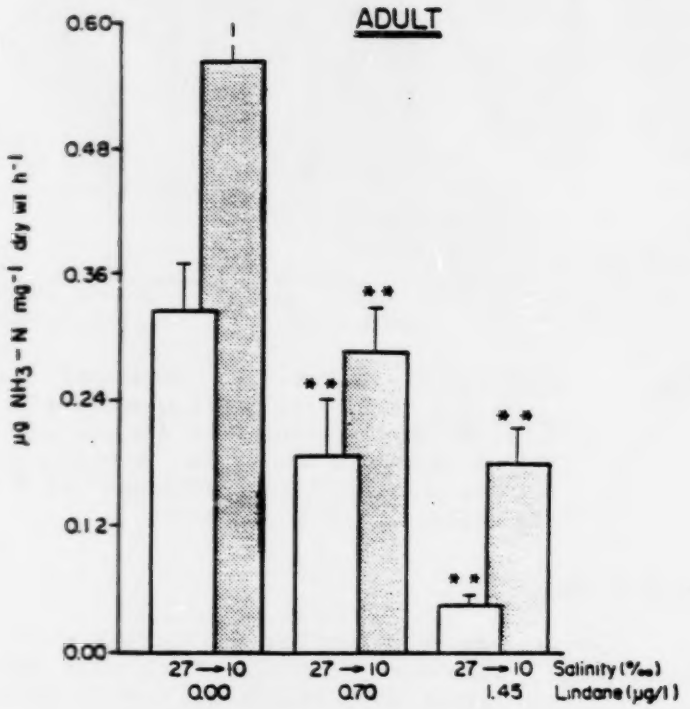

Fig. 7. Ammonia excretion rates of adult Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 21-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A double asterick indicates a significant difference ($\alpha = 0.01$) from the control at that salinity.

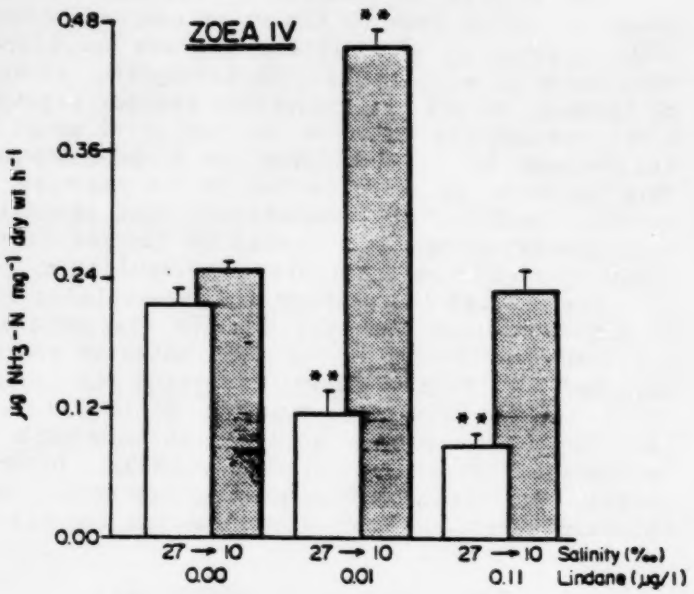

Fig. 8. Ammonia excretion rates of zoeal stage IV Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 16-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A double asterick indicates a significant difference (α = 0.01) from the control at that salinity.

When examining the osmoregulatory responses of euryhaline organisms, it is important to distinguish between short-term and long-term acclimatory mechanisms (Neufeld et al., 1980). In this study, responses were measured during the first 6 h after transfer of the animals from an isosmotic to a hypoosmotic condition and, therefore, reflect short-term acclimatory mechanisms.

The ability of adult E. depressus to adjust hemolymph osmotic concentration under hypoosmotic stress was significantly (α = 0.05) altered by exposure to 1.45 µg/L of lindane. Adjustments in membrane ion transport and water permeability are the major contributors to extracellular osmotic regulation in this euryhaline crustacean (Ruddy & Feng, 1975). This response, therefore, is an indication of a lindane-induced alteration of either one or both of these processes.

The pattern of hemolymph chloride ion regulation is known to follow that of the major cation, sodium (Lockwood, 1976; Spencer et al., 1979), and was specifically used in this study to monitor gill ion transport. Previous exposure to lindane, at all concentrations tested, significantly (α = 0.01) reduced the chloride ion ratios of adult E. depressus transferred to low salinity in a dose-dependent manner. This response of chloride ion ratios relative to hemolymph osmotic concentration demonstrates that specific ion transport processes can be affected by lindane exposure without altering overall extracellular osmoregulation.

The reduced sensitivity of extracellular osmoregulation to organochlorine exposure despite disruptions in specific ion transport processes has been noted in previous studies. Gill Na^+,K^+-ATPase of Cancer irroratus was inhibited both in vitro and in vivo with exposure to DDT; however, despite this fact, no changes occurred in hemolymph osmotic concentration (Neufeld & Pritchard, 1979). Other studies regarding the response of osmoregulatory mechanisms to organochlorine compounds have shown similar results. Exposure of

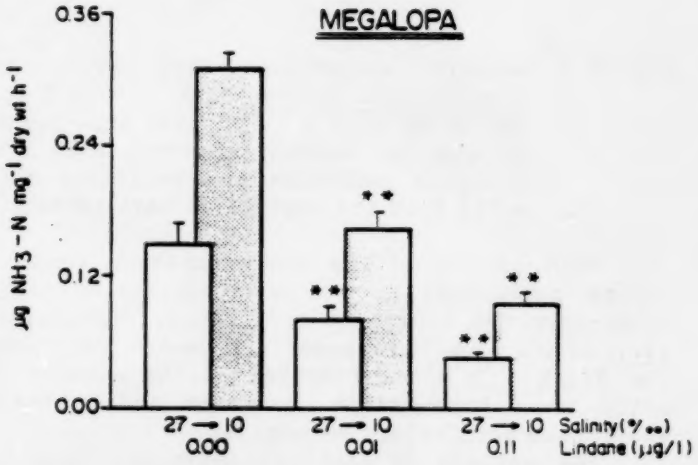

Fig. 9. Ammonia excretion rates of megalopal Eurypanopeus depressus at 27 o/oo S and upon transfer to 10 o/oo S after 21-d exposure to lindane at 27 o/oo S. Values represent mean ± SD. A double asterick indicates a significant difference (α = 0.01) from the control at that salinity.

Cancer magister and Hemigrapsus nudus to methoxychlor produced inhibition of Na^+,K^+-ATPase in the gills of these crabs, but osmotic regulation was not affected (Caldwell, 1974). PCB-exposed shrimp showed significant alterations in the concentration of major ions, but no particular osmotic change was observed (Nimmo & Blackman, 1972).

Various patterns of oxygen consumption have been associated with changing environmental salinity (Prosser, 1973; Lockwood, 1976). Changes in metabolic rates which accompany short-term hypoosmotic salinity acclimation primarily reflect differences in active ion transport and amino acid catabolism. Under normal conditions, respiratory control ensures that intracellular oxygen uptake is coupled to the need for ATP within the cell (Stryer, 1981). As long as the efficiency of ion uptake remains the same, increases in ATPase activity will increase oxygen consumption. In contrast, increases in nonessential amino acid metabolism, which accompanies low salinity acclimation in euryhaline crustaceans, has unpredictable effects on oxygen consumption. Depending on the degree of metabolic adjustment, associated with both a switch in energy substrate utilization and increasing active ion transport, various patterns of oxygen consumption may result. Pollutant exposure adds another level of unpredictability to the outcome of oxygen consumption measurements.

Short-term exposure to lindane concentrations of 0.70 µg/L and 1.45 µg/L significantly reduced respiration rates of adult E. depressus. This response was exhibited at both isosmotic and lowered salinities, indicating that in addition to its effects upon osmoregulation, lindane is affecting other metabolic processes.

Lindane concentrations of 0.01 µg/L and 0.11 µg/L increased the respiration rates of megalopae acclimated to the isosmotic salinity for adults. Under the same salinity and exposure conditions, zoeal respiration was unaffected. This result not only suggests an apparent physiological difference between the two larval stages, but also suggests a non-osmoregulatory effect of lindane exposure on E. depressus megalopae.

Exposure to 0.11 µg/L lindane and transfer to low salinity increased respiration rates of megalopae and zoeae relative to untreated larvae. These responses indicate lindane exposure had an affect upon metabolic processes associated with osmoregulation.

Increased ammonia excretion rates during adjustment to hypoosmotic environmental salinities is thought to be due to an increase in amino acid catabolism, which is the major effector of cell volume regulation (Emerson, 1969; Lockwood, 1976; Mangum & Towle, 1977; Spaargaren et al., 1982; Pressley & Graves, 1983). These changes in amino acid metabolism appear to be quite rapid. Burton and Feldman (1982) found that the size of the free amino acid pool of the intertidal copepod Tigriopus californicus responded within 3 h to changes in environmental salinity. In this study, therefore, ammonia excretion rates of crabs transferred to hypoosmotic conditions were used to reflect patterns of intracellular osmoregulation.

Lindane exposure reduced ammonia excretion rates at isosmotic and hypoosmotic salinities for adults and megalopae of Eurypanopeus depressus. Direct disruption of intracellular isosmotic regulation in lindane-exposed crabs may have occurred. However, because the ammonia excretion rates of crabs held in the isosmotic salinity were also decreased, this response to lindane exposure indicates a general non-osmoregulatory effect on amino acid catabolism.

Ammonia produced by amino acid catabolism at low salinities may act as a counter ion for energy-dependent influx of sodium (Campbell, 1973; Maetz, 1974; Silverthorn & Krall, 1975; Towle et al., 1976; Towle, 1981). It is possible that lindane, through interference with ionic regulation and Na^+-ATPase activity, is disrupting Na^+/NH_4^+ coupled transport mechanisms. Mangum et al., (1976) demonstrated that the rate of ammonia excretion at low salinities is correlated with the activity of transport ATPase. However, this effect cannot explain the reduced ammonia excretion of the crabs remaining at the isosmotic salinity.

Previous exposure of zoeae to a lindane concentration of 0.01 µg/L resulted in increased ammonia excretion rates upon transfer to low salinity. However, zoeae previously exposed to a higher lindane concentration of 0.11 µg/L did not exhibit altered ammonia excretion rates upon transfer to low salinity. Although this discrepancy cannot be explained by the present study, it does indicate a difference in the physiological/biochemical responses of zoeae to low-salinity transfer and lindane exposure compared to adult and megalopae.

The predicted ecological significance of sublethal lindane exposure are threefold: 1) disruption of osmoregulatory

mechanisms may limit the natural distribution of this species to area with less salinity fluctuations; 2) increased larval sensitivity to lindane exposure may reduce larval survival and recruitment in lindane-contaminated areas and subsequently shift the population age distribution to older individuals; and/or 3) eventually a more lindane-resistant population will be established or Eurypanopeus depressus will be eliminated from the affected area. The extrapolation of these results to other crustacean species is uncertain. Clearly, more field-oriented research is needed to assess the ecological significance of using physiological and biological indices as indicators of sublethal stress in marine biota.

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